GIBBERELLIN BIOSYNTHESIS:
Enzymes, Genes and Their Regulation

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ABSTRACT

The recent impressive progress in research on gibberellin (GA) biosynthesis has resulted primarily from cloning of genes encoding biosynthetic enzymes and studies with GA-deficient and GA-insensitive mutants. Highlights include the cloning of *ent*-copalyl diphosphate synthase and *ent*-kaurene synthase (formally *ent*-kaurene synthases A and B) and the demonstration that the former is targeted to the plastid; the finding that the *Dwarf-3* gene of maize encodes a cytochrome P450, although of unknown function; and the cloning of GA 20-oxidase and 3β-hydroxylase genes. The availability of cDNA and genomic clones for these enzymes is enabling the mechanisms by which GA concentrations are regulated by environmental and endogenous factors to be studied at the molecular level. For example, it has been shown that transcript levels for GA 20-oxidase and 3β-hydroxylase are subject to feedback regulation by GA action and, in the case of the GA 20-oxidase, are regulated by light. Also discussed is other new information, particularly from mutants, that has added to our understanding of the biosynthetic pathway, the enzymes, and their regulation and tissue localization.

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INTRODUCTION

The gibberellin (GA) hormones act throughout the life cycle of plants, influencing seed germination, stem elongation, flower induction, anther development, and seed and pericarp growth. Furthermore, they mediate environmental stimuli, which modify the flux through the GA-biosynthetic pathway. Regulation of GA biosynthesis is therefore of fundamental importance to plant development and its adaptation to the environment.

The last review of GA biosynthesis in this series, by Graebe (38), was followed by several reviews covering this topic (68, 75, 121). Graebe's article focused on GA-biosynthetic pathways in cell-free systems, characteristics of biosynthetic enzymes, and factors affecting GA production. He predicted accurately that the main topic of the next review in this series would be the cloning and characterization of genes for the GA-biosynthetic enzymes. Several of the enzymes have now been cloned, and the availability of their cDNAs is providing new and often unexpected information on the nature of these proteins. For example, some of the enzymes catalyze multiple steps in the pathway. It is also now possible to investigate the mechanisms by which GA biosynthesis is regulated in response to environmental and endogenous signals.

In light of these exciting advances, a new review on GA biosynthesis is appropriate. As is usual practice, we discuss the biosynthetic pathway, shown in Figures 1 and 2, in three sections according to the nature of the enzymes: terpene cyclases involved in *ent*-kaurene synthesis, monooxygenases, and di-
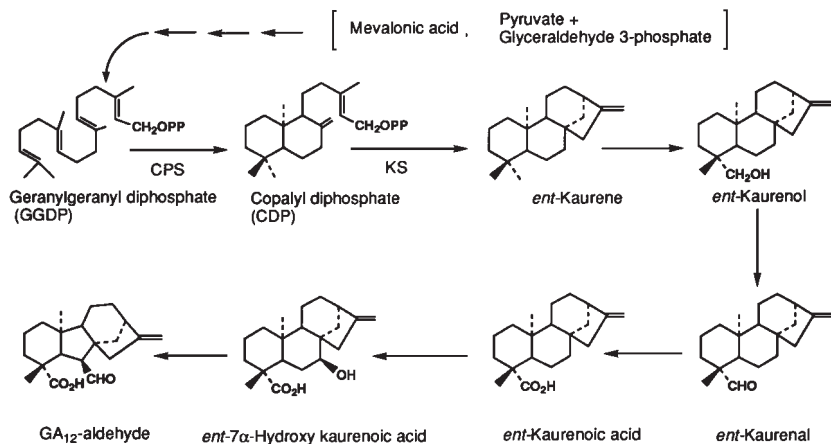
Figure 1   Early GA-biosynthetic pathway to GA$_{12}$-aldehyde. GGDP is produced in plastids by the isoprenoid pathway, originating from mevalonic acid or, possibly, pyruvate/glyceraldehyde 3-phosphate.

oxygenases. We also include discussions on regulation and sites of synthesis. We restrict ourselves to higher plants, because GA biosynthesis in other organisms, principally fungi and ferns, is not as well understood. However, future phylogenetic comparisons between GA biosynthesis genes in all organisms should be instructive in determining the origin of GAs. Much of the current progress on GA biosynthesis has come from work with mutants, which has been covered comprehensively in several reviews (98, 99, 101, 105) and is not dealt with specifically here. Some of the better characterized GA-deficient mutants, with the position of lesions, are given in Table 1, which also lists cDNA clones for biosynthetic enzymes.

ent-KAURENE SYNTHESIS

ent-Kaurene is synthesized by the two-step cyclization of geranylgeranyl diphosphate (GGDP) via the intermediate, *ent*-copalyl diphosphate (CDP). The enzymes that catalyze these reactions are referred to as the A and B activities, respectively, of *ent*-kaurene synthase (formerly *ent*-kaurene synthetase). However, we adopt the more logical nomenclature proposed by MacMillan (75). Thus, the conversion of GGDP to CDP is catalyzed by *ent*-copalyl diphosphate synthase (CPS) and of CDP to *ent*-kaurene by *ent*-kaurene synthase (KS). The biosynthesis of GGDP from mevalonic acid is common to many terpenoid pathways and is covered in the review by Chappell

Figure 2 Gibberellin-biosynthetic pathway from GA$_{12}$-aldehyde.

(13). Recently, a nonmevalonate pathway to isoprenoids, involving pyruvate and glyceraldehyde-3-phosphate, has been proposed in green algae (112). Such a pathway may operate in plastids of higher plants, given the difficulty in demonstrating the incorporation of mevalonate into isoprenoids in these organelles.

Table 1 Mutants and cDNA clones for GA-biosynthetic enzymes

Enzyme	Plant	Mutant	References	cDNA cloning	Data base
CPS	*Arabidopsis thaliana*	*ga1*	63	124	U11034
	Zea mays	*An1*	53	9	L37750
	Pisum sativum	*ls-1*	128	2	U63652
	Lycopersicon esculentum	*gib-1*	10	—	
KS	*Cucurbita maxima*	—	—	147	U43904
	A. thaliana	*ga2*	150	—	
	Z. mays	*d5*	48	—	
	L. esculentum	*gib-3*	10	—	
ent-Kaurene oxidase	*P. sativum*	*lh^i*	127	—	
	A. thaliana	*ga3*	150	—	
	Oryza sativa	*dx*	89	—	
Monoxygenase	*Z. mays*	*d3*	28	144	U32579
	P. sativum	*na*	49	—	
GA 20-oxidase	*C. maxima*	—	—	71	X73314
	A. thaliana	*ga5*	129	146	U20872
					U20873
					U20901
	A. thaliana	—	—	92	X83379
					X83380
					X83381
	P. sativum	—	—	77	X91658
				31	U70471
	P. sativum	—	—	73	U58830
	Phaseolus vulgaris	—	—	31	U70530
					U70531
					U70532
	O. sativa	—	—	137	U50333
	Spinacia oleracea	—	—	145	U33330
GA 3β-hydroxylase	*A. thaliana*	*ga4*	129	14	L37126
	Z. mays	*dl*	28	—	
	O. sativa	*dy*	59	—	
	P. sativum	*le*	50	—	
	Lathyrus odoratus	*l*	106	—	
GA 2-oxidases	*P. sativum*	*sln*	108	—	

CPS and KS were first separated by anion-exchange chromatography on extracts of *Marah macrocarpus* endosperm (23). There were also indications for the involvement of two enzymes from studies on GA-deficient mutants; work with cell-free extracts of young fruits suggested that the dwarf tomato mutants, *gib-1* and *gib-3,* have lesions at CPS and KS, respectively (10). Using an *ent*-kaurene oxidase inhibitor to estimate rates of *ent*-kaurene biosynthesis, a method first used with germinating barley grain (40), Zeevaart & Talon (150) demonstrated that the nonallelic *ga1* and *ga2* mutants of Arabidopsis

were both defective in *ent*-kaurene production, whereas *ga4* and *ga5* are blocked later in the pathway (129).

ent-Copalyl Diphosphate Synthase

Koornneef et al (63) constructed a fine structure genetic map of the Arabidopsis *GA1* locus using nine independent *ga1* alleles, three of which were made by fast neutron bombardment and the rest by treatment with ethylmethanesulfonate (EMS). Among the fast-neutron-generated mutants, *ga1-3* contains a large deletion (5 kb) and failed to recombine with the EMS-treated mutants (63). Sun et al (123) used *ga1-3* to clone the *GA1* locus by genomic subtraction. Cosmid clones containing wild-type DNA inserts spanning the deletion in *ga1-3* complemented the dwarf phenotype when integrated into the *ga1-3* genome by T-DNA transformation. *GA1* cDNA contains a 2.4-kb open reading frame, which was shown by functional analysis to encode CPS (124). *Escherichia coli* co-transformed with a bacterial GGDP synthase gene (12), and the *GA1* cDNA produced CDP, from which copalol was identified by combined gas chromatography–mass spectrometry (GC-MS) after alkaline hydrolysis. Although *ga1-3* contains a large deletion and genomic Southern analysis indicated that *GA1* is a single-copy gene, the mutant produces low amounts of GAs (150), suggesting that there are *GA1* homologues in Arabidopsis or there is an alternative pathway for *ent*-kaurene synthesis. A similar situation exists in maize, from which the *An1* (*Anther ear-1*) locus was cloned by transposon tagging (9). The predicted amino acid sequence of *An1* cDNA shares high sequence identity (51%, without transit peptide sequence) with that of the GA1 protein. A homozygous deletion mutant of *An1, an1-bz2-6923,* accumulated *ent*-kaurene to 20% of the wild-type content, indicating the presence of isoenzymes. Furthermore, a putative homologous cDNA, *An2,* was cloned by RT-PCR (8). At least two different *GA1* homologues have been obtained from tomato seedlings by RT-PCR using oligonucleotide primers based on Arabidopsis and maize CPS sequences (R Imai, personal communication). It appears, therefore, that leakiness of the *ga1-3* and *an1* deletion mutants is due to the presence of other CPSs.

The *Ls* locus of pea was shown to encode CPS. The *ls-1* dwarf mutant had reduced CPS activity in a cell-free system from immature seeds (127). Confirmation was obtained after cloning a CPS from pea by RT-PCR, its identity being confirmed by expression in *E. coli* of a glutathione S-transferase fusion protein with CPS activity (2). The *ls-1* mutation, produced by EMS treatment, is due to a G-to-A substitution at an intron-exon border that causes impaired splicing and a frameshift in the transcript (2).
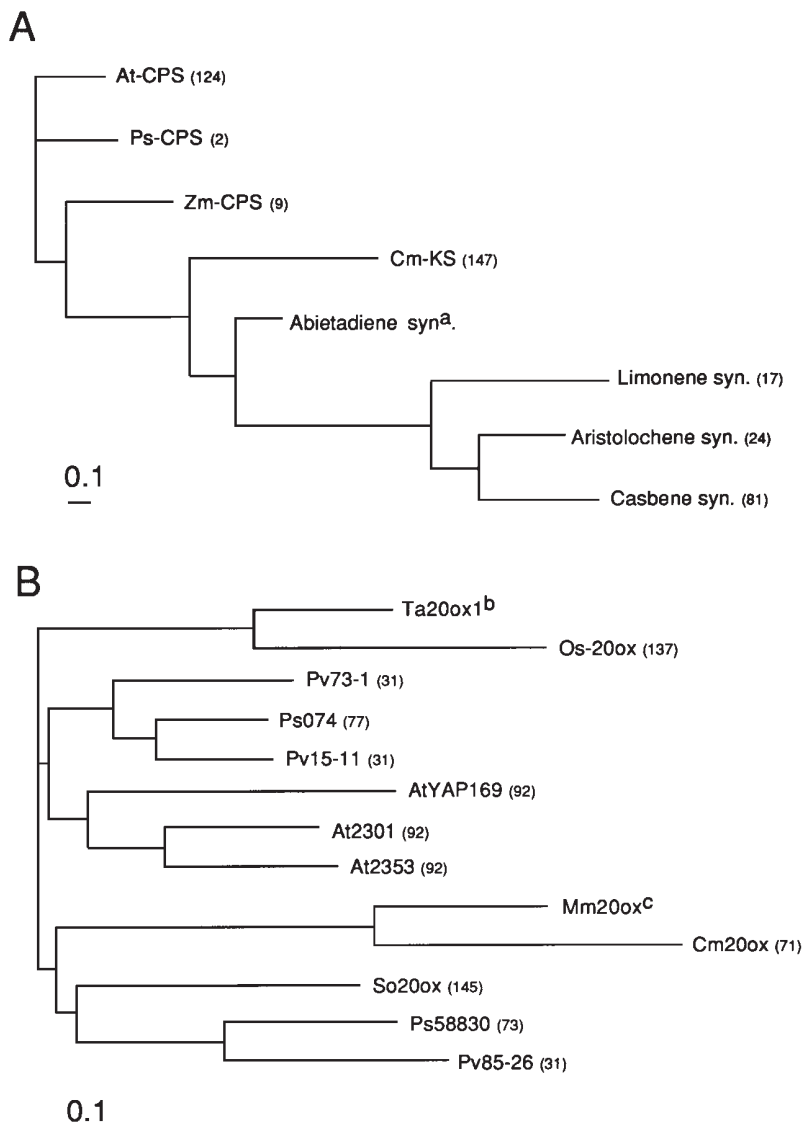
ent-Kaurene Synthase

ent-Kaurene synthase (KS) was purified from endosperm of pumpkin (*Cucurbita maxima*) (110), which is a rich source of GA-biosynthetic enzymes (38). The enzyme, which had a predicted M_r of 81,000, required divalent cations, such as Mg^{2+}, Mn^{2+}, and Co^{2+}, for activity and had an optimal pH range of 6.8–7.5 (110). The K_m for CDP was 0.35 μM. Purification of KS was quickly followed by its molecular cloning (147). PCR was used with degenerate oligonucleotides, designed from amino acid sequences of the purified protein, to produce a cDNA fragment for library screening. The isolated full-length cDNA was expressed in *E. coli* as a fusion protein, with maltose-binding protein, which converted [^{3}H]CDP to *ent*-[^{3}H]kaurene. The KS transcript is abundant in growing tissues, such as apices and developing cotyledons, and is present in every organ in pumpkin seedlings. Although it is difficult to compare mRNA abundance across species, it appears that KS is expressed at much higher levels than is CPS. Whereas CPS transcripts are undetectable in leaves of Arabidopsis (124) and pea (2) by northern blot analysis, requiring RNase protection assays (A Silverstone & TP Sun, personal communication) or RT-PCR, respectively, KS mRNA, though of low abundance, can be assayed in pumpkin leaves by northern hybridization (147). This is consistent with strict regulation of the first step of *ent*-kaurene synthesis from the abundant GGDP.

The deduced amino acid sequence of KS shares significant homology with other terpene cyclases (Figure 3*A*), with highest homology (51% amino acid similarity) with CPS from Arabidopsis and maize. It contains the DDXXD motif, which is conserved in casbene synthase (81), 5-*epi*-aristolochene synthase (24), and limonene synthase (17), and which is proposed to function as a binding site for the divalent metal ion-diphosphate complex (13). CPS lacks the DDXXD motif, consistent with its catalytic activity not involving cleavage of the diphosphate group.

Subcellular Localization of ent-Kaurene Synthesis

Although there has been evidence for *ent*-kaurene synthesis in plastids for over 20 years (reviewed in 38), unequivocal confirmation of this has been provided only recently. By precise use of marker enzymes to assess plastid purity and GC-MS to identify enzyme products, Aach et al (1) clearly demonstrated that CPS/KS activity (GGDP to *ent*-kaurene) is localized in developing chloroplasts from wheat seedlings and leucoplasts from pumpkin endosperm. Mature chloroplasts contained little activity. These results were supported by the recent cDNA cloning of CPS (124) and KS (147). The first 50 N-terminal amino acids of the GA1 protein (Arabidopsis CPS) are rich in serine and

A

B

Figure 3 Phylogenetic trees, produced using the PHYLIP package (J Felsenstein, University of Washington, Seattle), for (*A*) terpene cyclases, including *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS), and (*B*) GA 20-oxidases. Reference numbers are shown in parentheses. (Sources: [a]R Croteau et al, unpublished data; [b]NEJ Appleford, JR Lenton, AL Phillips & P Hedden, unpublished data; [c]DA Ward, J MacMillan, AL Phillips & P Hedden, unpublished data.)

threonine with an estimated pI of 10.2 (124). Such properties are common features of precursors of many chloroplast-localized proteins, such as the small subunit of Rubisco (54). The transit peptide is cleaved on entry into the plastid to produce a functional mature protein. Incubation of a ^{35}S-labeled Arabidopsis pre-CPS of 86 kDa with isolated pea chloroplasts resulted in transport into the chloroplasts and processing to a 76-kDa protein (124). The deduced amino acid sequence of KS also contains a putative transit peptide, although import into chloroplasts could not be demonstrated (147).

Plastids are the major site of production of GGDP, and most of the GGDP synthases cloned in plants have transit peptides for plastid transport (6). Localization of GGDP synthase from *Capsicum annum* in plastids has been demonstrated immunocytochemically (64). GGDP is a common precursor for many plastid-localized terpenoids, including carotenoids and the phytol side-chain of chlorophyll. Overexpression of phytoene synthase, which converts GGDP to phytoene, in transgenic tomato resulted in a lower chlorophyll content than in wild-type plants and a dwarf phenotype that was partially reversed by applying GA$_3$ (27). The endogenous GA concentrations in apical shoots of the transgenic plants were reduced to about 3% of that in wild-type shoots. If, as suggested (27), overproduction of phytoene has depleted GGDP content, resulting in reduced synthesis of GA and chlorophyll, the three pathways must share the same pool of GGDP and thus be interdependent.

MONOOXYGENASES

The highly hydrophobic *ent*-kaurene is oxidized by membrane-bound monooxygenases to GA$_{12}$. The enzymes require NADPH and oxygen and, on the basis of the early demonstration that *ent*-kaurene and *ent*-kaurenal oxidation are inhibited by carbon monoxide with reversibility by light at 450 nm (86), are all assumed to involve cytochrome P450. The involvement of cytochrome P450 in *ent*-kaurenoic acid 7β-hydroxylase in the fungus *Gibberella fujikuroi* has now also been shown (51). At least one of the enzymes (GA$_{12}$-aldehyde synthase) is associated with the endoplasmic reticulum in pea embryos and pumpkin endosperm (37), requiring transport of *ent*-kaurene, or perhaps a later intermediate, from the plastid.

Several GA-deficient dwarf mutants are defective in *ent*-kaurene oxidase activity. In pea, the *lh^i* (*lh-2*) mutation affects stem elongation and seed development (125–127). Cell-free extracts from immature *lh-2* seeds were deficient in *ent*-kaurene oxidase activity relative to wild-type seeds; the three steps from *ent*-kaurene to *ent*-kaurenoic acid were affected, suggesting that a single enzyme might catalyze these reactions (127). However, unequivocal verification

of this must await the availability of pure enzyme because a regulatory function for *Lh* cannot be excluded. The Tan-ginbozu mutant of rice (*dx*) is probably also deficient in *ent*-kaurene oxidase activity (89). Application of uniconazole, an *ent*-kaurene oxidase inhibitor, mimics the phenotype of Tan-ginbozu and produces endogenous GA concentrations similar to those in the mutant (89). Because of growth responses to applied *ent*-kaurene, Tan-ginbozu (85) and also *lh* (49) were thought previously to have lesions before *ent*-kaurene synthesis. The recent findings (89, 127) indicate that such application experiments may give misleading results.

Although less progress has been made with the monooxygenases than with the other enzymes, the recent cloning of the *Dwarf-3* (*D3*) gene of maize (144) should enable rapid progress in characterizing this group of enzymes. The *D3* gene, obtained by transposon tagging, was found, on the basis of its deduced amino acid sequence, to encode a member of a new class of cytochrome P450 monooxygenases with closest homology to sterol hydroxylases. Unfortunately, there is uncertainty about the step catalyzed by the D3 protein (BO Phinney, personal communication), although this should now be revealed by functional expression of the cDNA in a suitable heterologous system.

DIOXYGENASES

The enzymes involved in the third stage of the pathway are soluble oxidases that use 2-oxoglutarate as a co-substrate. These 2-oxoglutarate–dependent dioxygenases belong to a family of nonheme Fe-containing enzymes that have been the subject of several recent reviews (20, 94, 95). The enzymes show considerable diversity of function, but they are clearly related on the basis of conserved amino acid sequences.

The reactions known to be catalyzed by 2-oxoglutarate–dependent dioxygenases are shown as a network of pathways in Figure 2. Although they were originally delineated in developing seeds (38), both 13-hydroxylation and non-13-hydroxylation pathways have now been demonstrated in vegetative tissues (41, 60). The individual steps between GA_{12}-aldehyde and GA_3 and GA_8 were demonstrated in intact maize shoots by applying each isotopically labeled intermediate and identifying its immediate metabolite by GC-MS (30, 60). Both pathways were observed in a cell-free system from embryos/scutella of two-day-old germinating barley grain (41), although GA_4 was not identified. The dioxygenases in GA biosynthesis will now be discussed in detail, with particular emphasis on newer aspects not covered in the review by Lange & Graebe (68).

7-Oxidase

Oxidation at C-7 from an aldehyde to a carboxylic acid may be catalyzed by either dioxygenases or monooxygenases. Pumpkin endosperm contains both 7-oxidase activities (46), as does barley embryos/scutella (41). The dioxygenase activity from pumpkin has been partially purified and shown to have a very low pH optimum (72), whereas the monooxygenase is most active above pH 7 (46). The two types of activity also differ in their substrate specificities; the monooxygenase is specific for GA_{12}-aldehyde, whereas the soluble activity oxidizes several hydroxylated GA_{12}-aldehyde derivatives (46). The presence of both types of enzyme in a single tissue may indicate subcellular compartmentation of GA-biosynthetic pathways.

13- and 12α-Hydroxylases

As for GA 7-oxidase, both dioxygenase and monooxygenase forms of these hydroxylases have been described. The soluble, 2-oxoglutarate–dependent 13-hydroxylase detected in cell-free extracts from spinach leaves (36) is still the only example of a dioxygenase with this activity. 13-Hydroxylases in pumpkin endosperm (46, 69), developing pea embryos (52), and barley embryos/scutella (41) are of the monooxygenase type. The preferred substrate for the 13-hydroxylases is probably GA_{12}, although other GAs are hydroxylated to some extent. GA_{12}-aldehyde is 13-hydroxylated in embryo cell-free systems from *Phaseolus coccineus* (140) and *P. vulgaris* (128a), indicating that GA_{53}-aldehyde is an intermediate in the 13-hydroxylation pathway in these tissues. In the barley embryos/scutella system, GA_{15} and GA_{24} were 13-hydroxylated at very low rates compared with GA_{12}, while GA_9 was not metabolized (41). This result is similar to that found previously with microsomes from immature pea embryos (52), but in this case GA_{15} and GA_9 were hydroxylated only when their lactones were opened by hydrolysis. Although "late" 13-hydroxylation (on GA_9 or GA_4) can often be demonstrated, it may be relatively inefficient and accompanied by hydroxylation at other positions on the C and D rings (56). However, in some species, such as *Picea abies* (84), it would appear to be the major pathway.

Both forms of the 12α-hydroxylases are present in pumpkin seed, the monooxygenase hydroxylating GA_{12}-aldehyde (GA_{12} is not a substrate) (46), whereas the dioxygenase uses a variety of GA tricarboxylic acid substrates (69, 70). The monooxygenase has a low pH optimum and may thus catalyze part of the same pathway as the soluble 7-oxidase, for which 12α-hydroxyGA_{12}-aldehyde is a substrate. The soluble 12α-hydroxylase is sensitive to the presence of phosphate (69) and was, therefore, undetected in previous

studies in which phosphate, rather than Tris, buffer was used to extract the enzymes (45). Because phosphate removes Fe by precipitation, it would appear that the 12α-hydroxylase has an unusually high requirement for Fe^{2+}.

20-Oxidases

Formation of the C_{19}-GA skeleton requires successive oxidation of C-20 from a methyl group, as in GA_{12} or GA_{53}, through the alcohol and aldehyde, from which this C atom is lost as CO_2 (Figure 2). As discussed below, a single enzyme (GA 20-oxidase) can catalyze this reaction sequence, although the number of enzymes that are actually involved in vivo is unknown. A 2-oxoglu-tarate-dependent dioxygenase that converted GA_{53} to GA_{44} and GA_{19} was partially purified from 20-day-old developing embryos of *P. sativum* (67). Although the proportion of the two products remained constant throughout purification, it was uncertain whether a single enzyme catalyzed both steps. Clear evidence that GA 20-oxidases are multifunctional was obtained after purification of the enzyme to homogeneity from pumpkin endosperm (66). The enzyme converted GA_{12} to GA_{15}, GA_{24} and GA_{25}, and GA_{53} to GA_{44}, GA_{19} and GA_{17}, with a small amount of putative GA_{20} produced at high protein concentrations. GA_{12} was converted more efficiently than was GA_{53}. The production of the tricarboxylic acids, GA_{25} and GA_{17}, is characteristic of the pumpkin endosperm cell-free system (69) and indicates that the 20-oxidase in this tissue is functionally different from that encountered in other systems, which produce predominantly C_{19}-GAs (52, 128a).

The purification of the GA 20-oxidase from pumpkin was quickly followed by the cloning of a cDNA that encoded this enzyme (71). The cDNA was selected from an expression library, derived from developing embryos, using antiserum against a peptide sequence from the purified protein. As well as binding the antibodies, the expressed fusion protein was functionally active and catalyzed the same reactions as the native enzyme. The aldehyde interme-diate, GA_{24}, was converted to both GA_{25} and GA_9, although the latter was obtained in less than 1% yield. The presence of hydroxyl groups reduced the efficiency of conversion, by about 50% in the case of GA_{19} (13-hydroxylated) and 95% for GA_{23} (3β, 13-dihydroxylated), although small amounts of the C_{19}-GA product were detected in each case. The derived amino acid sequence corresponds to a protein of 43.3 kDa, which is close to that estimated for the native enzyme from gel filtration (44 kDa), and contains the conserved regions found in other plant dioxygenases.

The cloning of the GA 20-oxidase cDNA from pumpkin seeds enabled the isolation of homologous clones from other species. The first examples, from

Arabidopsis, were obtained independently in two laboratories (92, 146). Two GA 20-oxidase cDNAs were cloned from the *ga1-3* mutant utilizing PCR with degenerate primers designed from the conserved amino acid sequences; a third cDNA clone was found after scrutiny of the Data Base of Expressed Sequence Tags (92). Confirmation that all three cDNAs encoded GA 20-oxidases was obtained by demonstrating that the products of heterologous expression in *E. coli* converted GA_{12} to GA_9 and GA_{53} to GA_{20}, with GA_{12} the preferred substrate. Small amounts of the tricarboxylic acids, GA_{25} and GA_{17}, respectively, were formed but, in contrast to the pumpkin enzyme, the C_{19}-GAs were the major products. Thus, these enzymes appeared to be involved in the biosynthesis of active GAs. It was confirmed that at least one of the isozymes is active in vivo when a genomic clone encoding one of the GA 20-oxidases was isolated from Arabidopsis by probing a genomic library with the pumpkin 20-oxidase cDNA (146). The clone mapped tightly to the *GA5* locus, mutation of which results in semidwarfism (62) and a reduction in the concentrations of C_{19}-GAs (129). Expression of the GA 20-oxidase genes is tissue-specific, with transcripts detected, respectively, in stems/floral apices, floral apices/siliques, and siliques (92). The silique-specific 20-oxidase transcript is much more abundant than the others. The stem-specific gene corresponds to the *GA5* locus, mutation of which, in *ga5,* is due to a G to A substitution that introduces a premature stop codon (146). Although the mutant protein would be highly truncated and unlikely to be catalytically active, the *ga5* plant is semidwarfed and contains low amounts of C_{19}-GAs (129). It must be assumed, therefore, that other GA 20-oxidases, such as that expressed in the floral apex, supply GAs to the stem.

Gibberellin 20-oxidase cDNAs have been cloned from at least seven species, with multiple genes found in several of them. Their encoded amino acid sequences share a relatively low degree of sequence conservation, with amino acid identities ranging from 50–75%. The relationship between the sequences is shown in Figure 3*B*. With the exception of the enzyme from pumpkin seed, the proteins have very similar functions, converting 20-methyl GAs to the corresponding C_{19} lactones. It is notable that the enzyme cloned from developing cotyledons of *Marah macrocarpus* (J MacMillan & DA Ward, unpublished information) produces C_{19}-GAs despite its being most closely related to the pumpkin enzyme on the basis of sequence (Figure 3*B*). The structural differences that determine whether the 20-oxo intermediates are oxidized to C_{19}-GAs or to tricarboxylic acids are likely to be subtle. The 20-oxidases from pumpkin, *Marah,* and Arabidopsis prefer nonhydroxylated substrates to the 13-hydroxylated analogues. This is consistent with the types of GAs found in the tissues in which these enzymes are present. For example, GA_4, which is

not 13-hydroxylated, is the major GA in Arabidopsis shoots (129). In contrast, a GA 20-oxidase cloned from shoots of rice, in which 13-hydroxy C_{20}-GAs are the predominant forms (61), oxidizes GA_{53} more efficiently than it does GA_{12} (137).

There is evidence for the presence in shoot tissues of GA 20-oxidases with properties different from those that have been cloned so far. GA_{44} oxidase activity from spinach leaves was separated by anion-exchange chromatography from GA_{53} oxidase and GA_{19} oxidase activities, which co-eluted (35). These last two activities are induced by transfer of plants to long days, whereas GA_{44} oxidase activity is not photoperiod-sensitive (36). The spinach GA_{44} oxidase converts the lactone form of this GA (36), as do cell-free systems from pea shoots (38) and germinating barley embryos (41), whereas GA 20-oxidases from immature seeds require a free alcohol at C-20 for oxidation to occur (45, 52, 128a). Detailed studies with recombinant GA 20-oxidase, produced by expression of one of the Arabidopsis cDNAs in *E. coli,* revealed that this enzyme also required a free alcohol function and that oxidation of the alcohol was much slower than that of the methyl and aldehyde substrates (47). It seems likely, therefore, that a separate enzyme(s) with a high affinity for the 20-alcohols, perhaps as the lactones, exists in shoot tissues. An enzyme with similar properties to the Arabidopsis GA 20-oxidase has been cloned from spinach leaves (145). On the basis of the activity of the protein after expression in *E. coli* and its higher expression in long days than in short days, it would appear to correspond to the GA_{53} and GA_{19} oxidases that were observed in the leaf homogenates.

An unexpected difference between the spinach GA_{44} oxidase and the recombinant Arabidopsis GA 20-oxidase is in the stereospecific removal of a hydrogen atom during oxidation of the C-20 alcohol intermediates. It was shown, using GA_{15} or GA_{44} labeled stereospecifically with deuterium, that the Arabidopsis enzyme removes the *pro-R* H atom on conversion of the free alcohol to the aldehyde (142). In contrast, GA_{44}, as the lactone, is oxidized with loss of the *pro-S* H, by cell-free extracts of spinach leaves. This observation provides further evidence for the existence of a distinct lactone oxidase; the different stereochemistry of the reactions is presumably due to the fixed orientation of C-20 in the lactone as opposed to it assuming an energetically more favored conformation as the free alcohol.

3β-*Hydroxylases and Related Enzymes*

3β-Hydroxylation results in the conversion of the C_{19}-GAs GA_{20} and GA_9 to GA_1 and GA_4, respectively, in the final step in the formation of physiologi-

cally active GAs. There is now increasing evidence that, in common with GA 20-oxidases, certain GA 3β-hydroxylases may be multifunctional. An enzyme purified from developing embryos of *P. vulgaris* catalyzed 2,3-desaturation and 2β-hydroxylation reactions, in addition to 3β-hydroxylation (115, 116). GA_{20} and GA_9 were about equally reactive as substrates. A 3β-hydroxylase from the same source also epoxidized GA_5 to GA_6 (65). The enzyme could use non-2β-hydroxylated C_{19} (γ-lactone) GAs or 19–20 δ- lactone C_{20}-GAs as substrates (65), the latter presumably acting as structural analogues of the former, which are the natural substrates. An enzyme that 3β-hydroxylated GA_{15} to give GA_{37} was partially purified from pumpkin endosperm (72). It did not possess desaturase, although it was not tested with C_{19}-GAs.

The pumpkin GA 3β-hydroxylase has the typical properties of a 2-oxoglu-tarate-dependent dioxygenase (72). In particular, it was possible to demon-strate a 1:1 stoichiometry between the formation of hydroxy GA and succinate, once uncoupled oxidation of 2-oxoglutarate was subtracted. In contrast, al-though the *P. vulgaris* enzyme requires 2-oxoglutarate for activity, Smith et al (116) could find no evidence that this compound functioned as a substrate. They suggested that ascorbate may serve as the cosubstrate, as it does in the related enzyme, 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (22). However, 2-oxoglutarate is essential for full 3β-hydroxylase activity, whereas it serves no function for ACC oxidase activity. The nature of the *P. vulgaris* 3β-hydroxylase is unresolved.

The desaturase activity of 3β-hydroxylases provides the first step in the production of GA_3. After applying $[^3H]GA_5$ to immature seeds of apricot, de Bottini et al (19) obtained chromatographic evidence for the presence of GA_1, GA_3, and GA_6 in the products. Unequivocal evidence for conversion of GA_{20} to GA_3 via GA_5 was obtained in shoots of *Zea mays* (30), thus establishing a new biosynthetic pathway. There was no indication that GA_5 was converted to GA_1, a reduction that is without precedent in GA biosynthesis. The equivalent pathway for non-13-hydroxylated GAs was demonstrated in cell-free systems from immature seeds of *Marah* and apple (3). Enzyme activity present in both endosperm and developing embryos of *Marah* results in the conversion of GA_9 to GA_4 and 2,3-dehydroGA$_9$, which is further oxidized to GA_7 (75a). The *Marah* and apple systems have a marked preference for non-13-hydroxylated substrates; although both systems converted GA_5 to GA_3, GA_{20} was metabo-lized to GA_1 (3β-hydroxylation), GA_{29} (2β-hydroxylation), and GA_{60} (1β-hy-droxylation), but not to GA_5, by the *Marah* system and was unmetabolized by the apple preparation. The branch pathway from GA_{20} to GA_3 occurs also in barley embryos (41) and may be common, although not ubiquitous, in higher plants. The conversion of GA_5 to GA_3 has also been demonstrated in pea

shoots (93) and in a cell-free system from rice anthers (58), although because GA_5 is not formed in these systems (50, 57), the function of this activity is unclear.

The conversions of GA_5 to GA_3, and of 2,3-didehydroGA_9 to GA_7, are unusual reactions that are initiated by loss of the 1β-H (3). Hydrogen abstraction is accompanied by rearrangement of the 2,3 double bond to the 1,2 position and hydroxylation on C-3β. This enzymatic activity may also result in the 1β-hydroxylation of GA_{20} and GA_5, also observed in *Marah* (3). The enzyme that converts GA_5 to GA_3 requires 2-oxoglutarate, but not added Fe^{2+}, for activity and, in contrast with most other related dioxygenases, it is not inhibited by iron chelators (116). Its activity, however, is reduced by Mn^{2+} and other metal ions, the inhibition by Mn^{2+} being reversed by Fe^{2+}. It would appear that Fe is bound very tightly at the active site.

GA_3 and GA_1 formation in maize is apparently catalyzed by one enzyme (122). As well as affecting the conversion of GA_{20} to GA_1, the *dwarf-1* mutation reduces formation of GA_5 and the conversion of GA_5 to GA_3. If *Dwarf-1* is a structural gene, a single enzyme must catalyze all three reactions. In contrast, the *le* (3β-hydroxylation) mutation of pea was purported not to affect the conversion of GA_5 to GA_3 (93). This conclusion was based on equal growth responses of *Le* and *le* plants to applied GA_5, which was assumed to have no intrinsic biological activity. However, GA_5 is as active as GA_1 and GA_3 on *dwarf-1* maize shoots, despite no metabolism to GA_3 by this genotype (122).

The *ga4* mutation of Arabidopsis results in low amounts of GA_1 and GA_4 and an accumulation of GA_{20} and GA_9 in flowering shoots, indicating reduced 3β-hydroxylase activity (129). This conclusion was supported by an 85% reduction in the amount of GA_1 produced from labeled GA_{20} in *ga4* seedlings compared with those of Landsberg *erecta* or the *ga5* (20-oxidase) mutant (55). The *GA4* locus has been cloned by T-DNA insertion (14). The gene encodes a dioxygenase that has relatively low amino acid sequence identity with the GA 20-oxidases; it has 30% identity (50% similarity) with the Arabidopsis stem-specific 20-oxidase (GA5). Expression of the *ga4* cDNA in *E. coli* has confirmed that it encodes a 3β-hydroxylase (J Williams, AL Phillips & P Hedden, unpublished information). The preferred substrate for the recombinant enzyme is GA_9, for which the K_m is tenfold lower than that for GA_{20}. Thus, as with the Arabidopsis GA 20-oxidases, the presence of a 13-hydroxyl group reduces substrate affinity for the enzyme. The enzyme also epoxidizes the 2,3-double bond in GA_5 and 2,3-didehydroGA_9, and hydroxylates certain C_{20}-GAs, albeit with low efficiency. This activity could account for the presence of 3β-hydroxy C_{20}-GAs in Arabidopsis (129). However, there are undoubtedly other

GA 3β-hydroxylases active in this species. The original EMS-induced mutation (*ga4-1*) results in semidwarfism (62), and a reduction to about 30% of the normal content of 3β-hydroxy GAs (129). The mutant enzyme has an amino acid substitution, cysteine to tyrosine (14), that might allow a low level of activity. The mutant with the T-DNA insertion (*ga4-2*) is also a semidwarf, phenotypically similar to *ga4-1,* with very little likelihood of the mutant gene encoding an active 3β-hydroxylase. Residual growth in this mutant must, therefore, result from the action of other enzymes.

2β-*Hydroxylases and Related Enzymes*

Hydroxylation on C-2β results in the formation of inactive products and is, therefore, important for turnover of the physiologically active GAs. The natural substrates for these enzymes are normally C_{19}-GAs, although 2β-hydroxy C_{20}-GAs are also found in plant tissues, particularly where the concentration of C_{20}-GAs is high (76). 2β-Hydroxylases have been partially purified from cotyledons of *P. sativum* (118) and *Phaseolus vulgaris* (39, 117). There is evidence that, for both sources, at least two enzymes with different substrate specificities are present. Two activities from cotyledons of imbibed *P. vulgaris* seeds were separable by cation-exchange chromatography and gel-filtration (39). The major activity, corresponding to an enzyme of M_r 26,000 by size-exclusion HPLC, hydroxylated GA_1 and GA_4 in preference to GA_9 and GA_{20}, while GA_9 was the preferred substrate for the second enzyme (M_r 42,000).

Formation of 2-keto derivatives (GA catabolites) by further oxidation of 2β-hydroxy GAs (Figure 2) occurs in several species, but it is particularly prevalent in developing seeds (119) and roots (108) of pea. The conversion of GA_{29} to GA_{29}-catabolite in pea seeds was inhibited by prohexadione-calcium, an inhibitor of 2-oxoglutarate–dependent dioxygenase (87), indicating that the reaction is catalyzed by an enzyme of this type (108). Although the slender (*sln*) mutation of pea blocks both the conversion of GA_{20} to GA_{29} and of GA_{29} to GA_{29}-catabolite in seeds, the inability of unlabeled GA_{20} to inhibit oxidation of radiolabeled GA_{29}, and vice versa, indicated that the steps are catalyzed by separate enzymes (108). Furthermore, in shoot tissues, the slender mutation inhibits 2β-hydroxylation of GA_{20}, but not the formation of GA_{29}-catabolite. It was, therefore, proposed that *Sln* encodes a regulatory protein (108). Formation of the GA catabolites could be initiated by oxidation either at C-1 or C-2α. Although the reaction sequence is unknown, it is of interest that, after application of labeled GA_{20} to leaves of pea, labeled GA_{81} (2α-hydroxy GA_{20}) accumulates in the roots, together with the catabolites of GA_8 and GA_{29} (108).

GA_{81} is not formed from GA_{29} and must, therefore, be formed directly as a result of 2α-hydroxylase activity.

REGULATION OF GA BIOSYNTHESIS

The role of GAs as mediators of environmental stimuli is well established. Factors, such as photoperiod and temperature, can modify GA metabolism by changing the flux through specific steps in the pathway. More recent work has shown that GA biosynthesis is modified by the action of GA itself in a type of feedback regulation. The mechanisms underlying these regulatory processes can now be investigated as a result of the current advances in the molecular biology of GA biosynthesis.

Feedback Regulation

The presence of abnormally high concentrations of C_{19}-GAs in certain GA-insensitive dwarf mutants, such as *Rht3* wheat (5), *Dwarf-8* maize (29), and *gai* Arabidopsis (130), indicate a link between GA action and biosynthesis. In maize, there is a gene-dosage effect, with a 60-fold increase in the GA_1 content of homozygous *Dwarf-8* shoots, compared with wild-type and a 33-fold increase in the heterozygote. It was suggested that GA action results in the production of a transcriptional repressor that limits the expression of GA-biosynthetic enzymes (111). Mutants with impaired response to GA would lack this repressor and have elevated rates of GA production. Such plants normally contain semidominant mutations, which may result in a gain of function (91). It has been proposed that GA 20-oxidase is a primary target for feedback regulation (5, 18, 44). In addition to an elevated C_{19}-GA content, the GA-insensitive dwarfs often contain lower amounts of C_{20}-GAs than their corresponding wild-types, suggesting increased GA 20-oxidase activity. Conversely, overgrowth mutants, such as slender (*sln*) barley and *la cry^s* pea, that grow as if saturated with GA, even in its absence, contain reduced amounts of C_{19}-GAs and elevated content of C_{20}-GA GAs (18, 77). It appears that the slender mutation activates the GA signal transduction pathway, even in the absence of GA, and may thereby cause constitutive downregulation of GA 20-oxidase activity.

Further support for feedback regulation of GA 20-oxidase activity was provided by work with GA-biosynthesis mutants. Reduced concentrations of C_{20}-GAs, as well as a highly elevated GA_{20} content, are characteristics of most 3β-hydroxylase-deficient mutants, including *dwarf-1* maize (28), *le* pea (96), and *l* sweet pea (109). Treatment of the maize (44) or pea (77) mutants with 2,2-dimethylGA$_4$, a synthetic and highly bio-active GA, restored the

concentrations of GA_{20} and GA_{19} to those of wild types. Although the change in GA contents in the foregoing examples is associated with altered growth rates, the effect of GA action on GA metabolism is not a consequence of the change in growth rate. For example, there are numerous GA-insensitive dwarf mutants with normal GA contents, in which the mutation, normally recessive, is likely to affect processes that are not part of the primary response to GAs (99, 105).

With the availability of GA 20-oxidase cDNA clones, it has been possible to begin a molecular analysis of the feedback mechanism. Transcript levels for each of the three Arabidopsis GA 20-oxidase genes are much higher in the *ga1-3* (CPS-deficient) mutant than in Landsberg *erecta* and are very substantially reduced by treating the mutant with GA_3 (92). The reduction occurs within 1–3 h, long before a growth response is discernible (AL Phillips, D Valero & P Hedden, unpublished information), confirming that it is not related to growth-rate and indicating that the message is turned over rapidly. The level of mRNA for the stem-specific GA 20-oxidase is also higher in the *ga5* (GA-deficient) and *gai* (GA-insensitive) mutants than in the wild type (146). Treatment of *ga5* and, to a lesser extent, wild-type with GA_4, caused a reduction in GA 20-oxidase transcript levels, whereas treatment of *gai* resulted in a slight increase in 20-oxidase mRNA. Strong downregulation of GA 20-oxidase transcript levels by GA has also been observed in pea (77) and rice (137). Low endogenous GA concentration, as in mutants or after treatment with a biosynthesis inhibitor, consistently resulted in increased mRNA levels. Conversely, these levels were substantially reduced by application of GA. Furthermore, leaves of the slender (*la cry^s*) pea mutant contained only small amounts of 20-oxidase transcript, consistent with strong downregulation of gene expression (77).

Other enzymes in the pathway, particularly the GA 3β-hydroxylase, may also be subject to feedback regulation. Treatment of seedlings of the GA-deficient *na* mutant of pea with 2,2-dimethylGA$_4$ caused a slight reduction in the conversion of GA_{19} to GA_{20}, but a much greater reduction in GA_{20} metabolism (77). Furthermore, Chiang et al (14) found much more *GA4* (3β-hydroxylase) transcript in rosette leaves of *ga4* mutants than in Landsberg *erecta,* and that treatment with GA_3 reduced the amount of transcript within 8 h. It is possible that several enzymes are subject to regulation by GA; the identities of others may emerge when more enzymes of the pathway have been cloned.

Regulation by Light

The involvement of GAs in the photoperiod-induced bolting of long-day rosette plants is well documented (38). Transfer of *Silene armeria* plants from

short days (SD) to long days (LD) causes the GA_1 content to increase several-fold, particularly in the subapical region, with a decrease in GA_{53} content consistent with increased GA_{53} metabolism (131, 133, 134). In spinach (*Spinacia oleracea*), changes in GA concentrations (135) and enzyme activity in cell-free systems (36) on transfer from SD to LD are consistent with enhanced oxidation of GA_{53} and GA_{19} in LD. The activities of GA_{53} and GA_{19} 20-oxi-dases, now known to be the same enzyme (145), increase in the light and decrease in the dark (36). Furthermore, there are higher amounts of GA 20-oxidase mRNA in plants grown in LD than those in SD or in total darkness (145). It has been suggested that, in LD, there is sufficient GA 20-oxidase activity to raise the GA_1 concentration above the threshold required for stem extension (135). In fact, light appears to increase the total flux through the pathway, because *ent*-kaurene synthesis is also enhanced in LD in spinach and in *Agrostemma githago* (149). Although GA_{53} 20-oxidase activity is regulated by light, oxidation of GA_{44}, in the lactone form, remains at high, constant levels irrespective of light or dark treatment (36). As discussed earlier, this latter activity is probably because of another enzyme, which is not under light regulation.

Despite many attempts to implicate GA metabolism in phytochrome-mediated changes in growth rate, supporting evidence is sparse. Enhancement of GA_{20} 3β-hydroxylation by far-red light has been observed in lettuce (138, 139) and cowpea epicotyls (25, 31, 78, 79). In the latter case, higher GA_1 concentrations in plants grown in far-red light were due also to reduced 2β-hy-droxylation and were accompanied by heightened tissue responsiveness to GA (78, 80). However, in peas, enhanced shoot elongation by treatment with far-red-rich light was not associated with increased GA_1 content (100). There is also no evidence to suggest that dark-grown peas (34, 120, 143) or sweet peas (109) contain more GA_1 than light-grown plants. In fact, work with phytochrome- and GA-deficient mutants of pea indicates that growth inhibition by red light, which is mediated by phytochrome B, is due to altered responsiveness to GA, rather than to changes in the concentration of GA_1 (143). Several phytochrome-deficient mutants, such as the *ein* mutant of *Brassica rapa* (21) and the ma_3^R mutant of *Sorghum* (15, 16), have an overgrowth phenotype and were originally thought to contain abnormally high GA levels. Although the GA_1 content of these plants may be elevated (7, 104), this is apparently not the cause of their altered phenotype. Phytochrome B-deficient mutants of cucumber (74) and pea (143) contain comparable amounts of active GAs to the wild types, but show an enhanced response to GAs. In *Sorghum*, altered GA content is due to a shift in the phase of a diurnal fluctuation in GA concentrations (26). It was proposed that phytochrome deficiency disrupted

diurnal regulation of the conversion of GA_{19} to GA_{20}, resulting in a 12-h shift in the peaks of GA_{20} and GA_1 concentrations, whereas the pattern of fluctuation in the levels of GA_{12} and GA_{53} was unaffected.

Gibberellin metabolism is sensitive to light quantity. When pea seedlings were grown in low irradiance (40 μmol • m^{-2} • s^{-1}), GA_{20} concentration increased sevenfold compared with plants grown in high irradiance (386 μmol • m^{-2} • s^{-1}) (34), whereas in plants grown in the dark, the GA_{20} content was reduced to 25% of that in high irradiance. Moreover, the response of the seedling to exogenous GA_1 was heightened in the dark. These results indicate that the rate of GA 20-oxidation is sensitive to light fluence; with the cloning of GA 20-oxidases now reported for many species, the mechanisms underlying this process as well as the diurnal fluctuation in GA biosynthesis can be probed.

Regulation by Temperature

Induction of seed germination (stratification) or of flowering (vernalization) by exposure to low temperatures are processes in which GAs have been implicated. There are, however, few examples in which GAs have been shown unequivocally to mediate the temperature stimulus. The most extensively studied system is *Thlaspi arvense,* in which stem extension and flowering are induced by exposure to low temperatures followed by a return to higher temperatures (82). The same effect can be obtained without cold induction by application of GAs, the most active of those tested being GA_9 (83). In noninduced plants, *ent*-kaurenoic acid accumulates to high concentrations in the shoot tip, the site of perception of the cold stimulus, whereas after vernalization and return to high temperatures, the level of this intermediate falls within days to relatively low values (43). Metabolism of labeled *ent*-kaurenoic acid to GA_9 could be demonstrated in thermo-induced shoot tips, but not in noninduced material (42). Furthermore, microsomes from induced shoots metabolized *ent*-kaurenoic acid and *ent*-kaurene, but microsomes from noninduced shoots were much less active for both activities (43). Leaves, or microsomes extracted from leaves, from thermo-induced and noninduced plants metabolized *ent*-kaurenoic acid to the same extent. These results are consistent with regulation of *ent*-kaurenoic acid 7β-hydroxylase and, to a lesser degree, *ent*-kaurene oxidase by cold treatment in shoot tips of *Thlaspi.*

A change in GA content following vernalization was found in shoot tips of *Brassica napus,* in which GAs, including GA_1 and GA_3, accumulated during the cold period (148). The higher rates of GA production in vernalized, relative to nonvernalized, plants appeared to persist for 1–2 weeks after the return to

high temperatures. In contrast to these findings, there was no evidence to suggest that GAs were the signal for cold-induced flowering in *Raphanus sativus* (88) or *Tulipa gesneriana* (97).

Although the mechanism for thermo-induction of GA biosynthesis is not yet known, it has been suggested that cold treatment may allow increased rates of gene expression, possibly via demethylation of the promoters (11). Some circumstantial support for this theory was obtained by reducing DNA methylation in *Thlaspi* and late-flowering ecotypes of Arabidopsis by treatment with 5-azacytidine. Flowering times in noninduced plants were reduced in both cases. Confirmation of this theory must await the isolation and characterization of the 7β-hydroxylase gene.

SITES OF GA BIOSYNTHESIS

Developmental regulation of GA biosynthesis is determined mainly by changes in plant ontogeny during development and the tissue distribution of individual enzymes of the pathway. Work with legumes indicates that GA-biosynthesis occurs mainly in actively growing tissues, with leaves and internodes important sites (113, 114). Gibberellin 20-oxidase transcript levels are much higher in pea leaves than in internodes (32). The same 20-oxidase gene is expressed in shoots, young seeds, and expanding pods (32), but a different gene is expressed in developing cotyledons (73). Orthologues of the pea GA 20-oxidases, with similar patterns of gene expression, were cloned from French bean (32), and a third gene, which is expressed in developing cotyledons, leaves, and roots, was also detected in this species.

The tissue-specific expression of the GA 20-oxidase genes, also noted in Arabidopsis (92), has not been found for CPS (2, 124), KS (147), or GA 3β-hydroxylase (14). Whereas different GA 20-oxidase genes are expressed during pea seed development, the same CPS gene is expressed in a biphasic manner, corresponding with the two stages of GA production (2). The first phase, which results in the production of GA_1 and GA_3 (33, 103), is associated with seed development (126–128) and pod growth (33, 103). Both endosperm and testa are potential sites of synthesis (103). The second phase occurs in the developing embryo and has no known physiological function. As seeds approach maturity there is often an increase in 2-oxidation activities in embryos and testa (4, 119). The physiological significance of this deactivation mechanism is vividly demonstrated by the slender (*sln*) mutant of pea, which accumulates high concentrations of GA_{20} in the mature seed due to a lack of 2-oxidase activities (102, 107, 108). On germination, the GA_{20} is 3β-hydroxylated to GA_1, resulting in overgrowth of the first 10–12 internodes (102). An

intriguing phenotype was obtained by crossing slender with *na,* a GA-deficient dwarf, blocked at an intermediate step in the biosynthetic pathway, but not expressed in the developing seed (49). The double mutant is phenotypically slender to the six-leaf stage, but thereafter is severely dwarfed (108). Crossing slender with *le* (3β-hydroxylase-deficient) produced a dwarf from emergence, as did crossing with *lh^i,* which blocks at *ent*-kaurene oxidase in seeds (127) and prevents the accumulation of GA_{20} (108).

Gibberellin production in the pericarp of developing fruit may be important for fruit growth, particularly in the absence of seeds (132). Work with pea indicates that the presence of seeds stimulates GA biosynthesis in the pericarp (90, 141). Both seeds and 4-chloroindole-3-acetic acid, the proposed seed-derived signal, stimulated the conversion of GA_{19} to GA_{20} in pea pods. However, the finding that GA 20-oxidase transcript levels in pericarp from seeded pea fruit are much lower than in seedless fruit (32) does not support a regulatory role for GA 20-oxidation in fruit development in this species.

CONCLUDING REMARKS

Research on GA biosynthesis has reached an exciting stage. cDNA and genomic clones have been obtained for five types of enzyme, including members of each of the three enzymatic classes: cyclases, monooxygenases, and dioxygenases. The cloning of each enzyme in the pathway should be achieved within the next three to five years. The availability of clones is enabling significant advances in several directions. Enzymes can be prepared by heterologous expression in sufficient quantities for detailed studies on their structure and function, and for the production of antibodies. Promoter-reporter gene fusions, in situ hybridization, and immunolocalization can be used to determine cellular and subcellular sites of synthesis. It is possible to examine the regulation of individual enzymes at the transcript and protein levels. Such studies are already yielding important information on the regulation of GA 20-oxidase transcript abundance by GA action and by light, and on the developmental control of CPS and KS gene expression.

From a practical standpoint, it will be possible to manipulate GA production in transgenic plants by altering the expression of individual genes. This technology offers an alternative to the use of chemical growth regulators for the control of plant development. It also provides a means to alter the abundance of specific enzymes and thereby determine their contributions to the flux through the biosynthetic pathway. For example, overexpression of GA 20-oxidase cDNAs in Arabidopsis results in accelerated bolting, confirming that the activity of this enzyme is rate-limiting for this developmental process (53).

Experiments of this type may provide new insights into the role of GAs in plant development.

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